

## WHAT IS CLAIMED IS:

1. A double stranded polynucleotide molecule comprising a first coding strand and a second transcription template strand :
  - (a) said first coding strand comprising
    - (i) an expression cassette sequence which comprises, from 5' to 3', a promoter, a selected polynucleotide sequence the expression of which is controlled by said promoter, and a polyadenylation site, and
    - (ii) at least one first strand terminator sequence; and
  - (b) said second strand complementary to said first strand, wherein the portions of said second strand sequence complementary to the first strand terminator sequence do not impede transcription from the second strand sequence complementary to said first strand expression cassette, and wherein said second strand comprises at least one second strand terminator sequence which terminates transcription initiated on said second strand outside of the second strand sequence which is complementary to said first strand expression cassette.
2. The polynucleotide molecule according to claim 1, wherein said first strand terminator sequence is selected from the group consisting of a terminator sequence located 5' to said promoter, a terminator sequence located 3' to said polyadenylation site, a terminator sequence located on said first strand outside of said expression cassette sequence, and a terminator sequence located within the selected polynucleotide sequence of said expression cassette sequence.
3. The polynucleotide molecule according to claim 1 wherein said second strand terminator sequence is located on said second strand in a sequence which is not complementary to said expression cassette sequence.
4. The polynucleotide molecule according to claim 1, wherein said first strand further comprises a sequence complementary to a ribonucleolytic site.

5. The polynucleotide molecule according to claim 1, wherein said first strand further comprises a sequence complementary to a catalytic site.
6. The polynucleotide molecule according to claim 4, wherein said ribonucleolytic site is an RNA instability sequence.
7. The polynucleotide molecule according to claim 6, wherein said instability sequence is located on said first strand 3' to the second terminator sequence, 5' to the first terminator sequence, or within the coding sequence.
8. The polynucleotide molecule according to claim 2 wherein said first strand terminator sequence is located between 1 to 50 nucleotides 5' of said promoter.
9. The polynucleotide molecule according to claim 2 wherein said first strand terminator sequence is located on said first strand about 100 nucleotides 3' from said polyadenylation site.
10. The polynucleotide molecule according to claim 9 wherein said first strand terminator sequence is located on said first strand about 150 nucleotides 3' from said poly A site.
11. The polynucleotide molecule according to claim 2, wherein said first strand contains at least two terminators.
12. The polynucleotide molecule according to claim 2, wherein said first strand terminator sequence reduces unwanted transcription on said first strand.
13. The polynucleotide molecule according to claim 1 wherein the first strand does not have an inverted complementary repeat sequence of greater than 7 nucleotides.

14. The polynucleotide molecule according to claim 11, wherein said first strand terminator sequences are independently selected from the group consisting of a bacterial terminator, a bacteriophage terminator, a poly A site followed by an eukaryotic RNA pause site, a poly A site followed by an  $\alpha$ -globin terminator, a histone processing signal, a polynucleic acid sequence that provides a ribozyme cleavage site followed by said pause site; a ribonucleic acid cleavage site followed by said pause site, a rho-dependent terminator, a rho-independent terminator, and a circular single stranded padlock polynucleotide sequence torsionally linked to said polynucleotide molecule by hybridization between at least 10 consecutive nucleotides of said padlock and at least 10 consecutive nucleotides on said first strand.

15. The polynucleotide molecule according to claim 1 wherein second strand comprises more than one terminator sequence.

16. The polynucleotide molecule according to claim 1 further comprising at least one RNA instability sequence located on the second strand sequence in a position outside of the sequence complementary to the expression cassette sequence of said first strand.

17. The polynucleotide molecule according to claim 1 wherein said second strand does not have an inverted complementary repeat sequence of greater than 7 nucleotides.

18. The polynucleotide molecule according to claim 1 wherein said second strand does not have an inverted complementary repeat sequence of greater than 4 nucleotides.

19. The polynucleotide molecule according to claim 1 wherein said second strand terminator sequence is located 3' with reference to said first strand poly A site.

20. The polynucleotide molecule according to claim 1, wherein said second strand terminator sequence is located less than 200 nucleotides from the poly A site in the second sequence outside of the sequence that is complementary to said first strand expression cassette sequence.

21. The polynucleotide molecule according to claim 1 wherein said second strand terminator sequence is located 5' with reference to said first strand promoter.

22. The polynucleotide molecule according to claim 1 wherein said second strand terminator sequence is independently selected from the group consisting of a bacterial terminator, a bacteriophage terminator, a sequence comprising a ribozyme or ribonucleic acid cleavage site followed by an  $\alpha$ -globin terminator, a histone processing signal, a sequence comprising a ribonucleic acid cleavage site or ribozyme cleavage site followed by a eukaryotic RNA pause site, a rho-dependent terminator, a rho-independent terminator, and a circular single stranded padlock polynucleotide sequence torsionally linked to said polynucleotide molecule by hybridization between at least 10 consecutive nucleotides of said padlock and at least 10 consecutive nucleotides on said second strand.

23. The polynucleotide molecule according to claim 1 which is a polynucleotide vector.

24. The polynucleotide molecule according to claim 23 which further comprises a sequence which directs polynucleotide localization to the nucleus of a cell transfected with said molecule.

25. The polynucleotide molecule according to claim 1 wherein wobble nucleotides are altered to prevent the occurrence of an inverted complementary repeat.

26. The polynucleotide molecule according to claim 1 wherein said first and second strand sequences form a linear molecule.

27. The polynucleotide molecule according to claim 1 wherein said first and second sequences form a circular molecule.

28. The polynucleotide molecule according to claim 1 wherein said selected polynucleotide sequence encodes a protein.

29. The polynucleotide molecule according to claim 1 wherein said selected polynucleotide sequence has a biological activity.

30. The polynucleotide molecule according to claim 1 wherein said promoter is a weak promoter.

31. A double-stranded polynucleotide molecule wherein wobble bases in substantially all codons in the portion of the sequence that comprises a selected polynucleotide sequence are modified to encode the same amino acid sequence, but provide a nucleotide sequence which is substantially nonhomologous to a polynucleotide sequence present in a host cell.

32. A pharmaceutical composition comprising a polynucleotide molecule of any of claims 1-31, an optional agent that facilitates polynucleotide uptake in a cell, and a suitable pharmaceutically acceptable carrier.

33. A single stranded polynucleotide sequence selected from a first strand or second strand of a double stranded polynucleotide molecule of claim 1 through 32.

34. The polynucleotide sequence according to claim 33 wherein said terminator sequences are independently selected from the group consisting of a

bacterial terminator, a bacteriophage terminator, a sequence comprising a ribozyme cleavage site or a ribonucleic acid cleavage site followed by a eucaryotic RNA pause site or an  $\alpha$ -globin terminator, a histone processing signal, a rho-dependent terminator, and a rho-independent terminator.

35. A pharmaceutical composition comprising a polynucleotide molecule of any of claims 33-34, an optional agent that facilitates polynucleotide uptake in a cell, and a suitable pharmaceutically acceptable carrier.

36. A substantially single-stranded RNA molecule comprising a 5' end, a ribonucleotide sequence having a selected biological function when translated in a host cell, and a 3' end, said molecule being incapable of stably forming a double-stranded or partially double-stranded RNA molecule.

37. The molecule according to claim 36 wherein wobble nucleotides are altered to prevent the occurrence of an inverted complementary repeat.

38. The molecule according to claim 36, modified to prevent the extension of a hairpin at the 3' end.

39. The molecule according to claim 36 wherein said molecule contains no inverted complementary repeat sequences of greater than 7 nucleotides in length.

40. The molecule according to claim 36 wherein said molecule contains no inverted complementary repeat sequences of greater than 4 nucleotides in length.

41. The molecule according to claim 36 which comprises a cap at the 5' end of said molecule.

42. The molecule according to claim 38 wherein said modification comprises attached a chain terminator at the 3' end of said sequence.
43. The molecule according to claim 36 which comprises a Kozak sequence positioned in said sequence 5' to the 5' codon.
44. The molecule according to claim 36 which comprises a polyA tail.
45. The molecule according to claim 36 which comprises no polyA tail.
46. A substantially single-stranded RNA molecule wherein wobble bases in substantially all codons in the portion of the molecule sequence that comprises a selected polynucleotide sequence are modified to encode the same amino acid sequence, but provide a nucleotide sequence which is substantially nonhomologous to a polynucleotide sequence present in a host cell.
47. A pharmaceutical composition comprising a polynucleotide molecule of any of claims 36-45, an optional agent that facilitates RNA uptake in a cell, and a suitable pharmaceutically acceptable carrier.
48. A method for enhancing the efficiency of expression of a selected polynucleotide sequence in a host cell, said method comprising the step of transfecting said host cell with a double stranded polynucleotide molecule comprising a first coding strand and a second transcription template strand,
- (a) said first coding strand comprising
    - (i) an expression cassette sequence which comprises, from 5' to 3', a promoter, a selected polynucleotide sequence the expression of which is controlled by said promoter, and a polyadenylation site, and
    - (ii) at least one first strand terminator sequence; and

(b) said second strand complementary to said first strand, wherein the portions of said second strand sequence complementary to the first strand terminator sequence do not impede transcription from the second strand sequence complementary to said first strand expression cassette, and wherein said second strand comprises at least one second strand terminator sequence which terminates transcription initiated on said second strand outside of the second strand sequence that is complementary to said first strand expression cassette,

thereby inhibiting the formation of aberrant polynucleotide sequences transcribed from said polynucleotide molecule in said host cell.

49. The method according to claim 48, wherein said first strand terminator sequence is selected from the group consisting of a terminator sequence located 5' to said promoter, a terminator sequence located 3' to said polyadenylation site, a terminator sequence located on said first strand outside of said expression cassette sequence, and a terminator sequence located within the selected polynucleotide sequence of said expression cassette sequence.

50. The method according to claim 48, wherein said second strand terminator sequence is located on said second strand in a sequence which is not complementary to said first strand expression cassette sequence.

51. A method for treating a host subject comprising administering an effective amount of a pharmaceutical composition comprising a polynucleotide molecule of any of claims 1-31, an optional agent that facilitates polynucleotide uptake in a cell, and a suitable pharmaceutically acceptable carrier.



52. A method for enhancing the efficiency of expression of a selected polynucleotide sequence in a host cell, said method comprising the step of transfecting said host cell with a substantially single-stranded RNA molecule comprising a 5' end, a ribonucleotide sequence having a selected biological function when translated in a host cell, and a 3' end, said molecule being incapable of stably forming a double-stranded or partially double-stranded RNA molecule.

53. A method for treating a host subject comprising administering an effective amount of a pharmaceutical composition comprising a polynucleotide molecule of any of claims 36 through 45, an optional agent that facilitates RNA uptake in a cell, and a suitable pharmaceutically acceptable carrier.

54. A method for preventing the inadvertent shutting off or down regulation of a polynucleotide sequence present in a host cell transfected with a polynucleotide molecule containing a polynucleotide sequence homologous to said polynucleotide sequence, said method comprising the steps of :

administering an effective amount of a pharmaceutical composition comprising a polynucleotide molecule of any of claims 1 through 31 and 36 through 45, an optional agent that facilitates polynucleotide uptake in a cell, and a suitable pharmaceutically acceptable carrier.